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Letter to the Editor

Challenges of Quality Testing Program in Cellular Therapies

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Dear Editor,

In the last few decades, stem cells therapies and cancer immunotherapies expected to bring significant benefit to patients suffering a wide range of diseases and injuries. More than 350 clinical trials registered/completed for mesenchymal stem cells and more than 200 DC based clinical registered/completed so far. Still these therapies are in experimental stage. While generating clinical grade of mesenchymal stem cells or immune cells for clinical trials or for treatment, GMP practices ensure that the product is safe, consistent, effective and of good quality [1]. As time progresses, these cellular processing and manufacturing are more and more being performed in completely closed system? Allogenic transplantation of stem cells is also thrust area for research. Zhang J et al (2015) critically reviewed challenges and promises of allogeneic mesenchymal stem cells for use as a cell-based therapy [2].

Cell therapy based Product characterization should include testing for identity, purity, potency, viability and cell number. Additionally if require tumorigenicity and biocompatibility testing should be performed when suitable. The product should also include details of cell origin (autologous versus allogeneic), ability to initiate an immune response, , route of administration, duration of exposure, use of combination products, level of cell manipulation, ability to proliferate/differentiate etc [3].

During production of large number of mesenchymal stem cells or dendritic cells in is the case in cancer patients, these products have to be stored in liquid nitrogen. Before every infusion, cells should be analysed for viability and other quality testing parameters. Cell culture contaminants can be divided into two main categories: chemical contaminants such as impurities in media, sera, and water, endotoxins, plasticizers, and detergents, and biological contaminants such as bacteria, molds, yeasts, viruses, Mycoplasma, as well as cross contamination by other cells. While it is impossible to eliminate contamination entirely, it is possible to reduce its frequency and seriousness by gaining a thorough understanding of their sources and by following good aseptic technique

A clean room is any given contained space where provisions are made to reduce particulate contamination and control other environmental parameters such as temperature, humidity and pressure. Clean room has a controlled level of contamination that is specified by the number of particles per cubic meter at a specified particle size. All of the air delivered to a clean room passes through HEPA filters, and in some cases where stringent cleanliness performance is necessary, Ultra Low Particulate Air (ULPA) filters are used. Clean rooms are classified according to the number and size of particles permitted per volume of air. Normally for cell therapy lab, class 100- 10,000 clean rooms are sufficient to perform the cell processing. Clean room garments include boots, shoes, aprons, beard covers, bouffant caps, coveralls, face masks, frocks/lab coats, gowns, glove and finger cots, hairnets, hoods, sleeves and shoe covers. Garments may vary depending on type of clean room or work to be performed. Depending on work area, clean room factors need to evaluate before processing of cell therapy based product.

Bacterial Contamination

During differentiation or cell multiplication, there is a high possibility of bacterial contamination. We are able to see visible with naked eye with some indicators like turbidity, presence of particles visible in suspension, decline in pH. Some bacteria will grow slowly. All this leads to spoil the complete process. Therefore proper precaution by adding antibacterial agent like Penicillin/Streptomycin, Gentamycin Solution in the process may reduce chances of bacterial contamination. Before delivery of cell product to any patient/s gram staining should be followed in quality testing programmed. After negative to gram staining (Absence of bacteria) then cells product should be administrate to patient/s.

Bacterial Endotoxin

Endotoxin is lipopolysaccrides product of gram negative bacteria. Endotoxin is small, stable, bacterially-derived hydrophobic molecules which can easily contaminate lab ware and whose presence can significantly impact cell product. Therefore it is very important to measure endotoxin level before infusing cell product to patients. The limulus amebocyte lysate (LAL) has been widely used for the detection of Gram-negative bacterial endotoxin with the help of gel clot assay or quantitative assay by plate reader.

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Mycoplasma

Contamination in cell culture is common problem while growing cell therapy product. Moreover these are resistant to most of the antibiotics [4]. Most common sources for Mycoplasma contamination are culture reagents like serum, primary tissue isolate or cross contamination with other culture. Proper filter sterilization of serum, sterilization of media or reagents may reduce chances of Mycoplasma contamination. It is very difficult to detect Mycoplasma by microscopy. A common method to detect Mycoplasma is by using a Luminometer or it may also be detected by polymerase chain reaction.

Fungal/Mold contamination

Contamination of stem cell culture is easily the most common problem encountered in cell culture laboratories. Molds are also eukaryotic microorganisms in the kingdom of Fungi that grow as multicellular filaments called hyphae. Due to fungal or Mold contamination pH remain stable at initial stages of contamination, then rapidly increases as the culture become more heavily infected and becomes turbid. Under microscopy, the mycelia usually appear as thin, wisp-like filaments, and sometimes as denser clumps of spores. Fungizone is commonly used antibiotic used in cell culture to avoid fungal contamination however it can sometimes kills the cell products too.

Flow Cytometry Characterization

Flow cytometry is widely used for characterization of cell therapy products. This single platform is useful to characterize the different properties of the cells in a single time. Before introducing any cell products into patients, one should know cell viability of final product to be administered. There are number of viability dyes available in the market, like Propidium iodide (PI), 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI), 7-amino-actinomycin D (7-AAD), Sytox dyes, amines dye, vital dyes. However, the most widely used dyes are PI and 7aad dye for assessing cell products viability. Both are DNA binding dyes that are membrane impermeant, meaning that the dyes will only bind dead cells. Surface marker characterization studies are very important before infusing cell products to patients. Mesenchymal stem cells should be positive for CD105, CD73, and CD90 and negative for CD45, CD34, CD14 or CD11b, CD79a or CD19 [5]. Dendritic cells should positive for CD80, CD83, CD86 and negative for CD14 [6].

Monitoring Outcomes of Cell Based Therapy

After administration of cells to patients, the most important aspect of the cell based therapy is monitoring efficacy of the treatment. In dendritic cell based therapy ELISPOT assay, CFC assay, Tetramer analysis, qRT-PCR, T cell assessment like CD4/CD8 analysis can be done to monitor immune response [7]. For assessment of mesenchymal stem cells in chronic kidney failure, clinical assessment and laboratory test like urea, creatinine and GFR levels before and after stem cells infusion can be monitored for six months and beyond. Gabr H and Zayed A (2015) found that the infusion of mesenchymal stem cells leads to statistically significant improvement in blood urea, creatinine levels and GFR levels in chronic renal failure patients [8]. Wang L et al (2013) studied umbilical cord MSC therapy for Patients with Active Rheumatoid Arthritis. They evaluated serum levels of inflammatory chemokines/cytokines and lymphocyte subsets in peripheral blood to monitor clinical outcomes. The study showed serum levels of tumor necrosis factor-alpha and interleukin-6 were decreased and the percentage of CD4+CD25+Foxp3+ regulatory T cells of peripheral blood were increased [9]. Study of Bhansali et al (2009) showed autologous bone marrow stem cells transplantation can leads to β-cell regeneration, leading to reduction in insulin requirement in patients with T2DM requiring high doses of insulin [10].

Proper monitoring and quality control programmes can lead to accurate delivery of dendritic cells or stem cells at target sites. Adequate delivery of high quality stem cells will help us understand proper mechanisms and efficacy of the therapy, enabling us to continue on the right direction, the aim being ensuring reproducibility and developing a proper path for regulatory approval and commercialization.

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